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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Reissue Applicants	:	Hiroyuki Nakane, Chikara Ohto, Shinichi Ohnuma, Kazutake Hirooka, Tokuzo Nishino
Reissue Serial No.	:	09/902,651
Reissue Application Filed	:	July 12, 2001
Patent No.	:	5,935,832
Issued	:	August 10, 1999
Title	:	FARNESYL DIPHOSPHATE SYNTHASE
Examiner	:	STEADMAN, David J.
Art Unit	:	1656

Box REISSUE
Assistant Commissioner for Patents
Washington, D.C. 20231

SUBSTITUTE REISSUE APPLICATION DECLARATION BY THE INVENTORS

We, Hiroyuki Nakane, Chikara Ohto, Shinichi Ohnuma, Kazutake Hirooka and Tokuzo Nishino, hereby declare that:

1. Each inventor's residence, mailing address and citizenship are stated below next to their name.

2. We believe the inventors named below to be the original and first inventors of the subject matter which is described and claimed in patent number 5,935,832 (the '832 patent), granted August 10, 1999 and for which a reissue patent is sought on the invention entitled "Farnesyl Diphosphate Synthase," the specification of which was filed on July 12, 2001 as reissue application number 09/902,651 and was amended on July 12, 2001, March 18, 2005, November 7, 2005, March 31, 2006, June 2, 2006, June 23, 2006, December 22, 2006, June 29, 2007, October 20, 2007, June 3, 2008, March 6, 2009, February 16, 2010, July 13, 2010, and August 9, 2010.

3. We have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

4. We acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56.

5. We hereby claim the foreign priority benefit under 35 U.S.C. 119 of the Japanese application listed below.

Foreign Application Priority Information:

Serial No.	**Filing Date**
Japan 8-213211	Jul. 24, 1996

6. We verily believe the original patent to be partly invalid, for the reason described below: by reason of patent claim 1 being indefinite, or patent claim 9 failing to further limit patent claim 1.

7. At least one error upon which reissue is based is described below.

Claim 1 of the '832 patent is directed to a mutant prenyl diphosphate synthase, and the claim is reproduced below.

Claim 1. A mutant prenyl diphosphate synthase having a modified amino acid sequence, wherein

said mutant diphosphate synthase comprises an aspartic acid-rich domain having the sequence, $D_1D_2X_1X_2(X_3X_4)D_3$, in region II of said mutant prenyl diphosphate synthase,

wherein each of D_1, D_2, and D_3 denote an aspartic acid residue; X_1, X_2, X_3, and X_4 are each independently any amino acid and X_3 and X_4 are each optionally independently present in the aspartic acid rich domain, and wherein

said mutant prenyl disphosphate synthase comprises (1) at least one amino acid substitution, said at least one amino acid substitution located at least one amino acid position selected from (a) an amino acid between D_1 and the amino acid residue at the fifth position upstream of D_1 and (b) the amino acid residue located

one amino acid position upstream of D_3; (2) at least one additional amino acid inserted between D_3 and the first amino acid upstream of D_3; or a combination of (2) and (3);

wherein said mutant prenyl diphosphate synthase synthesizes prenyl diphosphate which is shorter than prenyl diphosphate synthesized by a corresponding wild-type enzyme.

Claim 9 of the '832 patent depends on patent claim 1, and is reproduced below.

Claim 9. A mutant prenyl diphosphate synthase according to claim 1 wherein at least one amino acid selected from phenylalanine at position 77, threonine at position 78, valine at position 80, histidine at position 81, and isoleucine at position 84 has been substituted by another amino acid, and/or two amino acids have been inserted in between isoleucine at position 84 and methionine at position 85 in the geranylgeranyl diphosphate synthase as set forth in SEQ ID NO: 1, wherein the phenyl alanine at position 77 has been replaced with tyrosine, the threonine at position 78 has been replaced with phenylalanine or serine, the valine at position 80 has been replaced with isoleucine, the histidine at position 81 has been replaced with leucine or alanine, or the isoleucine at position 84 has been replaced with leucine; or proline and serine have been inserted in between the isoleucine at position 84 and the methionine at position 85.

In patent claim 1, the recitation of "a combination of (2) and (3)" renders the claim indefinite because patent claim 1 does not recite what item (3) is, and the specification does not disclose what item (3) is. The '832 patent is partly invalid under 35 U.S.C. §112, second paragraph, due to the indefiniteness of patent claim 1.

Even though patent claim 9 depends on patent claim 1, the embodiment of the mutant prenyl diphosphate synthase according to patent claim 9 wherein "proline and serine have been inserted in between the isoleucine at position 84 and the methionine at position 85" of the geranylgeranyl diphosphate synthase set forth in SEQ ID NO:1 (please see the last three lines of patent claim 9 reproduced above, or the last two lines of claim 9 printed in the '832 patent) is NOT covered by patent

claim 1. Applicants note that in the amino acid sequence of geranylgeranyl diphosphate synthase set forth in SEQ ID NO:1, position 82 corresponds to D_1, position 83 corresponds to D_2 and position 86 corresponds to D_3 of the sequence, $D_1D_2X_1X_2(X_3X_4)D_3$, recited in patent claim 1. The embodiment of the mutant wherein "proline and serine have been inserted in between the isoleucine at position 84 and the methionine at position 85" of the geranylgeranyl diphosphate synthase set forth in SEQ ID NO:1 is **not covered by** patent claim 1 of the '832 patent because the two amino acids are inserted between position 84 and position 85, and such an insertion is

NOT (1) at least one amino acid substitution, said at least one amino acid substitution located at least one amino acid position selected from (a) an amino acid between D_1 and the amino acid residue at the fifth position upstream of D_1 and (b) the amino acid residue located one amino acid position upstream of D_3; and

NOT (2) at least one additional amino acid inserted between D_3 and the first amino acid upstream of D_3, recited in patent claim 1.

Regarding limitation (2) of patent claim 1, at least one additional amino acid inserted between D_3 and the first amino acid upstream of D_3, applicants note that the first amino acid upstream of D_3 is position 85 if the amino acid sequence of SEQ ID NO:1 is considered as the wild type prenyl diphosphate synthase. In contrast, in the embodiment of the mutant according to patent claim 9, the proline and serine are inserted between position 84 and position 85, not between position 85 and position 86 as required by limitation (2) of patent claim 1.

As a result, the '832 patent is partly invalid at least by reason of patent claim 9 failing to further limit the subject matter claimed in patent claim 1 as required by 35 U.S.C. §112, fourth paragraph.

8. Furthermore, inadvertant typographical errors are present in the '832 specification. These errors are corrected in the amendments provided below.

A. At col. 1, line 15, please delete the "s" from "unit[s]". The paragraph containing this text is reproduced below, with the correction identified.

> Of the substances having important functions in organisms, many are biosynthesized using isoprene (2-methyl-1,3-butadiene) as a constituent unit[s]. These compounds are also called isoprenoids, terpenoids, or terpenes, and are classified depending on the number of carbon atoms into hemiterpenes (C5), monoterpenes (C10), sesquiterpenes (C15), diterpenes (C20), sesterterpenes (C25), triterpenes (C30), tetraterpenes (C40), and the like. The actual biosynthesis starts with the mevalonate pathway through which mevalonic acid-5-diphosphate is synthesized, followed by the synthesis of isopentenyl diphosphate (IPP) which is an active isoprene unit.

B. At col. 1, line 45, please delete the "plants" and at col. 1, line 46, after "in" please insert "plants." The paragraph containing this text is reproduced below, with the corrections identified.

> There are Z type and E type condensation reactions. Geranyl diphosphate is a product of E type condensation and neryl diphosphate is of Z type condensation. Although, the all-E type is considered to be the active form in farnesyl diphosphate and geranylgeranyl diphosphate, the Z type condensation reaction leads to the synthesis of natural rubber, dolichols, bactoprenols (undecaprenols), and [plants] various polyprenols found in plants. They are believed to undergo the condensation reaction using the phosphate ester bond

energy of the pyrophosphate and the carbon backbone present in the molecule and to produce pyrophosphate as the byproduct of the reaction.

C. At col. 2, line 4, please delete "[geraniols and that isomer nerol belonging]" and substitute "geraniol and its isomer, nerol, belonging " therefor. At col. 2, line 5, after "monoterpens" please insert "that." The paragraph containing this text is reproduced below, with the corrections identified.

> Furthermore, via the biosynthesis of these active-form isoprenoids, a vast number of kinds of compounds that are vital to life have been synthesized. Just to mention a few, there are cytokinins that are plant hormones and isopentenyl adenosine-modified tRNA that use hemiterpenes as their precursor of synthesis, [geraniols and that isomer nerol belonging] geraniol and its isomer, nerol, belonging to monoterpens that are the main components of rose oil perfume and a camphor tree extract, camphor, which is an insecticide. Sesquihormones include juvenile hormones of insects, diterpenes include a plant hormone gibberellin, trail pheromones of insects, and retinols and retinals that function as the visual pigment precursors, binding components of the purple membrane proteins of highly halophilic archaea, and vitamin A.

D. At col. 3, line 45, please delete "[(DDXX(XX)D)]" and substitute "$(D_1D_2X_1(X_2X_3)X_4D_3)$" therefore. At col. 3, line 56, please delete the "[have

not been]" and substitute "are" therefor. At col. 3, line 57, please delete the "[that induce mutation]" and substitute "that include mutations" therefor. At col. 3, lines 58-59, please delete "[to be in the short chain-length side]" and substitute with having a shorter chain length. The paragraph containing this text is reproduced below, with the corrections identified.

> It has been found out that of the two aspartic acid-rich domains that have been proposed based on the amino acid sequence of the prenyl diphosphate synthase, the amino acid residue located at the fifth position in the N-terminal direction from the conserved sequence I [(DDXX(XX)D)] ($D_1D_2X_1(X_2X_3)X_4D_3$) (wherein X denotes any amino acid, and the two X's in the parentheses may not be present) of the aspartic acid-rich domain in the amino-terminal side is responsible for controlling the chain length of the reaction product. Hence, a method has been invented that controls the reaction product for the purpose of lengthening the chain length of the reaction product [Japanese patent application No. 8-191635 filed on Jul. 3, 1996 under the title of "A Mutant Prenyl Diphosphate Synthase"]. The enzyme produced using the method enables production of reaction products that have several chain lengths. However, methods [have not been] are not known [that induce mutation] that include mutation of geranylgeranyl diphosphate synthase to control the reaction products [to be in the short chain-length side] having a shorter

chain length in order to produce farnesyl diphosphate.

E. At col. 4, line 2, please delete the "[owned by the]" and substitute "exhibited by" therefor. The paragraph containing this text is reproduced below, with the corrections identified.

> It is an object of the invention to establish a process for producing farnesyl diphosphate synthases by modifying amino acid sequences of prenyl diphosphate enzymes. A new enzyme that is more stable or that has a high specific activity more adaptable to industrial application would make it possible to obtain immediately a mutant prenyl diphosphate synthase or the gene thereof that produces farnesyl diphosphate and that retains the property [owned by the] exhibited by the prenyl diphosphate synthase prior to mutation.

F. At col. 4, line 11, please delete "[(DDXX(XX)D)]" and substitute "$(D_1D_2X_1(X_2X_3)X_4D_3)$" therefore. The paragraph containing this text is reproduced below, with the corrections identified.

> From the information on the nucleotide sequence of the gene of the geranylgeranyl diphosphate synthase of the mutant Sulfolobus acidocaldarius (S. acidocaldarius), it was clarified that out of the two Aspartic acid-rich domains that have been proposed based on the analysis of the amino acid sequence of prenyl diphosphate synthases, the amino acid residues within the aspartic acid-rich domain conserved sequence I [(DDXX(XX)D)] $(D_1D_2X_1(X_2X_3)X_4D_3)$ at the amino terminal side or the five amino acid residues to the N-terminal side from the amino terminal of said conserved sequence I are involved in the control of chain length of the reaction products.

G. At col. 4, line 23, please delete "[(DDXX(XX)D)]" and substitute "($D_1D_2X_1(X_2X_3)X_4D_3$)" therefor. At col. 4, line 26, please delete "[at the position in the N-terminal direction from D of the C-terminal of said aspartic acid-rich domain]" and substitute "one amino acid position downstream of D2" therefor. The paragraph containing this text is reproduced below, with the corrections identified.

> at least one amino acid residue selected from (a) the amino acid residues in between the amino acid residue located at the fifth position in the N-terminal direction from D of the N-terminal and the amino acid residue located at the first position in the N-terminal direction from D of said N-terminal of the aspartic acid-rich domain [DDXX(XX)D] ($D_1D_2X_1(X_2X_3)X_4D_3$) (wherein X sequence denotes any amino acid, and the two X's in the parentheses may not be present) present in region II, and (b) the amino acid residue located one amino acid position downstream of D2 [at the position in the N-terminal direction from D of the C-terminal of said aspartic acid-rich domain] has been substituted by another amino acid, and/or

H. At col. 4, line 32, please delete "[amino acid residues located at the first position in the N-terminal direction from D of the C-terminal and D of said C-terminal]" and substitute "first amino acid downstream of D_2 and the first amino acid upstream of D_3" therefor. The paragraph containing this text is reproduced below, with the corrections identified.

> additional amino acid(s) have been inserted in between the first amino acid downstream of D_2 and the first amino acid upstream of D_3 [amino acid residues located at the first position in the N-terminal direction from D of the C-terminal and D of said C-terminal] of said aspartic acid-rich domain.

I. At col. 5, line 36, please delete "[(DXX(XX)D)]" and substitute "($D_1D_2X_1(X_2X_3)X_4D_3$)" therefore. The paragraph containing this text is reproduced below, with the corrections identified.

It has been proposed that there are five conserved regions in the amino acid sequence of a prenyl diphosphate synthase (one subunit in the case of a heterodimer) [A. Chem et al., Protein Science Vol. 3, pp. 600-607, 1994]. It is also known that of the five conserved regions, there is an aspartic acid-rich domain conserved sequence I [[DDXX(XX)D]] $\underline{D_1D_2X_1(X_2X_3)X_4D_3}$ (wherein X denotes any amino acid, and the two X's in the parentheses may not be present) in region II. Although there is also an aspartic acid-rich domain indicated as "DDXXD" in region V, the aspartic acid-rich domain used to specify the modified region of the amino acid sequence of the present invention is the one present in region II, and this domain is termed as the aspartic acid-rich domain I as compared to the aspartic acid-rich domain II present in region V.

J. At col. 6, line 6, please delete "[(DDXX(XX)D)]" and substitute "$\underline{(D_1D_2X_1(X_2X_3)X_4D_3)}$" therefore. At col. 6, line 8, please delete "[at the position in the N-terminal direction from D of the C-terminal of said aspartic acid-rich domain]" and substitute "<u>one amino acid position downstream of D2</u>" therefor. The paragraph containing this text is reproduced below, with the corrections identified.

In accordance with the present invention, in the amino acid sequence of a prenyl diphosphate synthase, at least one amino acid residue selected from (a) the amino acid residues in between the amino acid residue located at the fifth position in the N-terminal direction from D of the N-terminal and the amino

acid residue located at the first position in the N-terminal direction from D of said N-terminal of the aspartic acid-rich domain [DDXX(XX)D] $\underline{D_1D_2X_1(X_2X_3)X_4D_3}$ (wherein X sequence denotes any amino acid, and the two X's in the parentheses may not be present) present in region II, and (b) the amino acid residue located <u>one amino acid position downstream of D2</u> [at the position in the N-terminal direction from D of the C-terminal of said aspartic acid-rich domain] has been substituted by another amino acid, and/or

K. At col. 6, line 16, lease delete "[amino acid residues located at the first position in the N-terminal direction from D of the C-terminal and D of said C-terminal]" and substitute "<u>first amino acid downstream of D_2 and the first amino acid upstream of D_3</u>" therefor. The paragraph containing this text is reproduced below, with the corrections identified.

an additional amino acid(s) have been inserted in between the <u>first amino acid downstream of D_2 and the first amino acid upstream of D_3</u> [amino acid residues located at the first position in the N-terminal direction from D of the C-terminal and D of said C-terminal] of said aspartic acid-rich domain.

L. At col. 7, line 67, please delete "[biding]" and substitute "<u>binding</u>" therefor. The paragraph containing this text is reproduced below, with the corrections identified.

It is known that the distance between the sequence of the ribosome [biding] <u>binding</u> site (GGAGG and similar sequences thereof) and the initiation codon ATG is important as the sequence regulating the ability of synthesizing protein from mRNA. It is also well known that a terminator (for example, a vector containing rrn PT_1 T_2 is commercially available from Pharmacia)

that directs transcription termination at the 3'-end affects the efficiency of protein synthesis by a recombinant.

M. At col. 9, line 14, please delete "[a]" and substitute "an" therefor. At col. 9, line 16, please delete "[prrenyl]" and substitute "prenyl " therefor. The paragraph containing this text is reproduced below, with the correction identified.

> By using the method of producing the mutant prenyl diphosphate synthase obtained by the present invention, the mutant prenyl diphosphate synthase derived from [a] an archaea may be created that is more stable and thus easier to handle and that produces [prrenyl] prenyl diphosphate. Furthermore, there is also expected a creation of the farnesyl diphosphate-producing mutant prenyl diphosphate synthase that has the property of the prenyl diphosphate synthase prior to mutation (for example, salt stability or stability in a wide range of pH) added thereto.

N. At col. 10, line 4, please delete "[Geranylaeranyl]" and substitute "Geranylgeranyl" therefor. The paragraph containing this text is reproduced below, with the corrections identified.

> Construction of a Plasmid Containing the Gene for [Geranylaeranyl] Geranylgeranyl Diphosphate Synthase

O. At col. 10, line 57, please delete "[TATT-31]" and substitute "TATT-3' " therefor. The paragraph containing this text is reproduced below, with the corrections identified.

> Introduction of the mutation (F77Y, T78S, V80I, I84L, 84PS85) was effected using two nucleotides. First, mutation was introduced as mentioned in Example 3 using the oligonucleotide
>
> 5'-GTTCTTCATACTTATTCGCTTATTCATGATAG
>
> [TATT-31] TATT-3' (SEQ ID No: 7) and a transformant was prepared in accordance with Example 4, and furthermore mutation was introduced into the plasmid thus obtained using the oligonucleotide
>
> 5'-ATTCATGATGATCTTCCATCGATGGAT CAAGAT-3' (SEQ ID No: 8).

P. At col. 11, line 24, please delete "[H2O]" and substitute "H_2O " therefor. The paragraph containing this text is reproduced below, with the corrections identified.

> [H2O] H_2O 5 µl

Q. At col. 11, line 56, please delete "[H2O]" and substitute "H_2O " therefor. The paragraph containing this text is reproduced below, with the corrections identified.

> [H2O] H_2O make to a final volume of 10 µl

R. At col. 12, line 35, please delete "[ATATCATG-31]" and substitute "ATATCATG-3' " therefor. The paragraph containing this text is reproduced below, with the corrections identified.

F77Y, T78F, H81L:
5'-TATTTCCTTGTGCTTGATG
[ATATCATG-31] ATATCATG-3' (SEQ ID No: 11)

9. All errors corrected in this reissue application arose without any deceptive intention on the part of the applicants.

10. We hereby declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon, or any patent to which this declaration is directed.

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